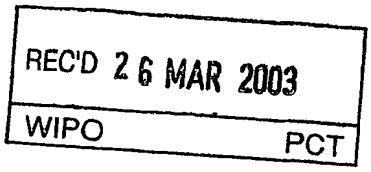
INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

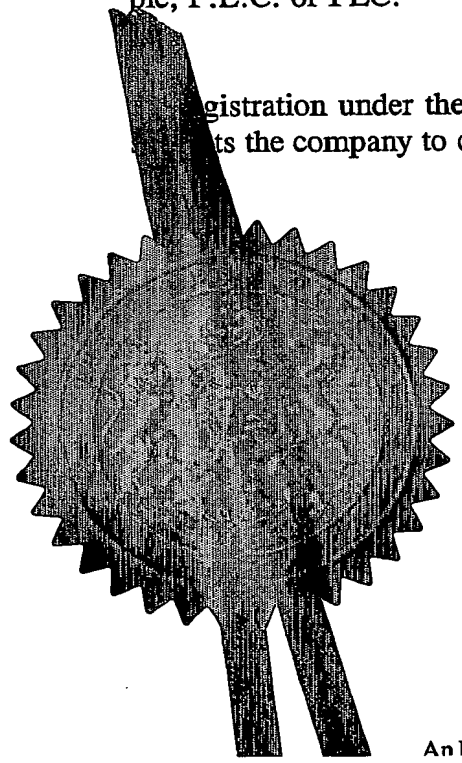


I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

gistration under the Companies Act does not constitute a new legal entity but merely ts the company to certain additional company law rules.



Signed

Dated 28 November 2002

BEST AVAILABLE COPY

Patents Form 1/77

Patents Act 1977
(Rule 16)

06 MAR 2002

The Patent Office

07MAR02 E701299-1 D00524
P01/7700 0.00-0205281.9

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office
Cardiff Road
Newport
Gwent NP10 8QQ

1.	Your reference	4-32343P1
2.	Patent application number *(The Patent Office will fill in this part)*	0205281.9
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	NOVARTIS AG LICHTSTRASSE 35 4056 BASEL SWITZERLAND
	Patent ADP number *(if you know it)*	7125487001
	If the applicant is a corporate body, give the country/state of its incorporation	SWITZERLAND
4.	Title of invention	Organic compounds
5.	Name of your agent *(If you have one)*	
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	B.A. YORKE & CO. CHARTERED PATENT AGENTS COOMB HOUSE, 7 ST. JOHN'S ROAD ISLEWORTH MIDDLESEX TW7 6NH
	Patents ADP number *(if you know it)*	1800001

6.	If you are declaring priority from one ore more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing (day/month/year)
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing (day/month/year)
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *(see note (d))*	Yes		

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	**11**
Claim(s)	**7**
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	**ONE**
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application

Signature: B. A. Yorke & Co.

Date: **06 March 2002**

12. Name and daytime telephone number of person to contact in the United Kingdom: **Mrs. E. Cheetham**
020 8560 5847

Warning

After an application for a patent has been filed. the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the united Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *Once you have filled in the form you must remember to sign and date it.*

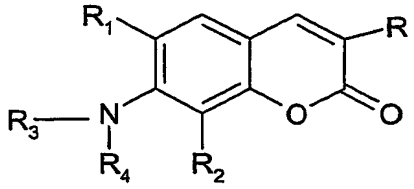
e) *For details of the fee and ways to pay please contact the Patent Office.*

DUPLICATE

Organic Compounds

The present invention relates to novel coumarine derivatives, their preparation, their use as markers and compositions containing them.

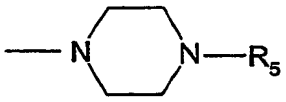
More particularly the invention provides a compound of formula I



I

wherein

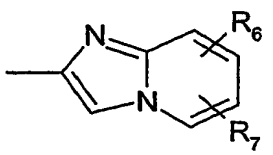
either R_1 and R_2 are both hydrogen and either R_3 and R_4, independently, are H, CH_3, $^{11}CH_3$, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, n being 2, 3 or 4, or R_3 and R_4, together with the nitrogen atom to which they are attached, form a group of formula



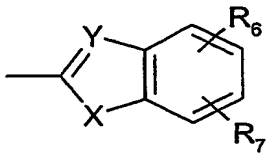
wherein R_5 is H, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, CH_3, $^{11}CH_3$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, n being as defined above,

or one of R_1 and R_2 is hydrogen and the other, together with R_3, forms a $-(CH_2)_m-$ bridge, m being 2 or 3, and R_4 is H, CH_3, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $^{11}CH_3$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, and

R is a group of formula



or



wherein X is O, S or NR_8, R_8 being H, CH_3, $^{11}CH_3$, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$ (n being as defined above), Y is CH or N and R_6 and R_7, independently, are H, NO_2, F, ^{18}F, $O(CH_2)_nF$, $O(CH_2)_n{}^{18}F$, Cl, CN, ^{11}CN, OCH_3, $O^{11}CH_3$, I, ^{123}I, $O(CH_2)_nI$ or $O(CH_2)_n{}^{123}I$ (n being as defined above),

in free base or acid addition salt form, for use as a marker.

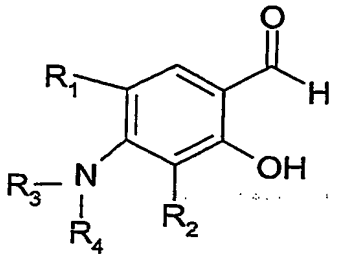
The compounds of formula I, with the exception of the following compounds:

7-Dimethylamino-3-(1-methyl-1H-benzoimidazol-2-yl)-chromen-2-one
3-(1H-Benzoimidazol-2-yl)-7-dimethylamino-chromen-2-one
3-(6-Chloro-benzothiazol-2-yl)-7-dimethylamino-chromen-2-one
3-Benzothiazol-2-yl-7-dimethylamino-chromen-2-one
3-Benzooxazol-2-yl-7-dimethylamino-chromen-2-one
3-Benzooxazol-2-yl-7-methylamino-chromen-2-one
3-(5-Chloro-benzooxazol-2-yl)-7-dimethylamino-chromen-2-one

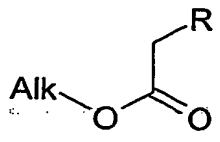
have never been disclosed in the literature and are also part of the present invention.

In a further aspect, the invention provides a process for the production of the compounds of formula I and their salts, comprising the steps of

a) for the production of a compound of formula I wherein R_3, R_4, R_5, R_6, R_7 and R_8 are different from $^{11}CH_3$, $(CH_2)_n{}^{18}F$, $(CH_2)_n{}^{123}I$, ^{18}F, $O(CH_2)_n{}^{18}F$, ^{11}CN, $O^{11}CH_3$, ^{123}I and $O(CH_2)_n{}^{123}I$, reacting a compound of formula II with a compound of formula III



II



III

wherein R_3 and R_4 as well as R_5 in R_3 and R_4; R_6 and R_7 in R; and R_8 in X are different from $^{11}CH_3$, $(CH_2)_n{}^{18}F$, $(CH_2)_n{}^{123}I$, ^{18}F, $O(CH_2)_n{}^{18}F$, ^{11}CN, $O^{11}CH_3$, ^{123}I and $O(CH_2)_n{}^{123}I$, and Alk is (C_{1-4})alkyl, or

b) for the production of a compound of formula I wherein at least one of R_6 and R_7 is $O^{11}CH_3$, reacting a compound of formula I wherein at least one of R_6 and R_7 is OH with $I^{11}CH_3$ and a base, or

c) for the production of a compound of formula I wherein at least one of R_6 and R_7 is $O(CH_2)_n{}^{18}F$, respectively $O(CH_2)_n{}^{123}I$, reacting a compound of formula I wherein at least one of R_6 and R_7 is $O(CH_2)_nOTs$ or $O(CH_2)_nOMs$ with $^{18}F^{\ominus}$, respectively $^{123}I^{\ominus}$, or

d) for the production of a compound of formula I wherein at least one of R_6 and R_7 is ^{18}F, reacting a compound of formula I wherein at least one of R_6 and R_7 is NO_2 or halogen, with $^{18}F^{\ominus}$, or

e) for the production of a compound of formula I wherein at least one of R_6 and R_7 is ^{123}I, reacting a compound of formula I wherein at least one of R_6 and R_7 is Bu_3Sn, with ^{123}I and hydrogen peroxide, or

f) for the production of a compound of formula I wherein at least one of R_6 and R_7 is ^{11}CN, reacting a compound of formula I wherein at least one of R_6 and R_7 is OSO_2CF_3 with [^{11}C]cyanide, or

g) for the production of a compound of formula I wherein at least one of R_3, R_4, R_5 and R_8 is $^{11}CH_3$, reacting a compound of formula I wherein at least one of R_3, R_4, R_5 and R_8 is hydrogen, with $^{11}CH_3I$, or

h) for the production of a compound of formula I wherein at least one of R_3, R_4, R_5 and R_8 is $(CH_2)_n{}^{18}F$, respectively $(CH_2)_n{}^{123}I$, reacting a compound of formula I wherein at least one of R_3, R_4, R_5 and R_8 is $(CH_2)_nOTs$ or $(CH_2)_nOMs$ with $^{18}F^{\ominus}$, respectively $I^{\ominus}$,

and recovering the resulting compound of formula I in free base form or in form of an acid addition salt.

The reactions can be effected according to known methods, for example as described in the Examples.

Working up the reaction mixtures and purification of the compounds thus obtained may be carried out in accordance to known procedures.

Acid addition salts may be produced from the free bases in known manner, and vice-versa.

Compounds of formula I in free base or acid addition salt form, hereinafter referred to as agents of the invention, exhibit valuable properties as histopathological staining agents, imaging agents and/or biomarkers, hereinafter "markers".

More particularly the agents of the invention are useful as markers for labeling pathological structures such as intraneuronal neurofibrillary tangles and extracellular ß-amyloid plaques, e.g. in the brain of patients with Alzheimer's disease (see Example 5).

The agents of the invention are therefore useful for the early diagnosis and prevention of Alzheimer's disease and for monitoring the effectiveness of therapeutic treatments of Alzheimer's disease.

The advantages of assessing amyloid and neurofibril deposition in vivo and non-invasively using markers capable of labeling these structures have been reported e.g. in WO 00/10614.

In accordance with the above, the present invention provides a composition for labeling histopathological structures in vivo or in vitro, comprising an agent of the invention.

In a further aspect, the present invention provides a method for labeling histopathological structures in vitro or in vivo, which comprises contacting brain tissue with an agent of the invention.

Said brain tissue comprises for example ß-amyloid plaques and/or neurofibrillary tangles.

Contacting the brain tissue with the agent of the invention is for example effected by administering the agent of the invention to a patient, e.g. a patient with Alzheimer's disease.

The method of the invention may comprise a further step aimed at determining whether the agent of the invention labeled the target structure.

If the agent of the invention is a non-radioactive compound of formula I, said further step may be effected by observing the target structure using fluorescence microscopy.

If the agent of the invention is a radioactive compound of formula I, said further step may be effected by observing the target structure using positron emission tomography (PET) or single photon emission computed tomography (SPECT).

Labeling histopathological structures in vitro is effected, for example, for detecting histopathological hallmarks of Alzheimer's disease.

Labeling histopathological structures in vivo is effected, for example, for diagnosing Alzheimer's disease in a patient or for monitoring the effectiveness of a therapeutic treatment of Alzheimer's disease.

The following examples illustrate the invention.

Example 1: 3-Benzothiazol-2-yl-7-[4-(2-fluoro-ethyl)-piperazin-1-yl]-chromen-2-one

200 mg (0.793 mmol) 4-[4-(2-Fluoro-ethyl)-piperazin-1-yl]-2-hydroxy-benzaldehyde and 164 mg (1 eq.) benzothiazol-2-yl-acetic acid methyl ester are heated to reflux for 3h in 5 mL benzene and 2.5 mL acetonitrile, in the presence of 0.157 mL (2 eq.) piperidine. The reaction mixture is allowed to reach room temperature and the precipitate filtered off, washed with diethylether and dried under high vacuum to yield 90 mg (55%) of desired product as a yellow powder (melting point: 246°C).
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.95 (s, 1H); 8.03, 7.97 (2d, 2H); 7.53 (D, 1H); 7.50, 7.39 (2t, 2H); 6.88 (dd, 1H); 6.78 (d, 1H); 4.62 (dt, 2H); 3.45 (t, 4H); 2.78 (dt, 2H); 2.70 (t, 4H).

The starting materials are prepared as described hereafter:

3-[4-(2-Fluoro-ethyl)-piperazin-1-yl]-phenol
1 g (5.61 mmol) 3-Piperazin-1-yl-phenol and 0.5 mL (1.25 eq) 1-bromo-2-fluoroethane are stirred 20 h at 60°C in 5 mL DMF, the reaction mixture allowed to reach room temperature and evaporated, and the residue column chromatographed (silica gel, ethyl acetate/ petroleum ether 9:1) to yield 600 mg (48%) of the desired product as a brown oil.

4-[4-(2-Fluoro-ethyl)-piperazin-1-yl]-2-hydroxy-benzaldehyde
600 mg (2.675 mmol) 3-[4-(2-Fluoro-ethyl)-piperazin-1-yl]-phenol are dissolved in 8 mL DMF and cooled to 0°C. 0.27 mL (1.1 eq) $POCl_3$ is added dropwise within 2 min. and the reaction mixture stirred for an additional 5 min. before being allowed to reach room temperature, and then heated and stirred for 3 h at 90°C. The reaction mixture is evaporated and the residue extracted with water and ethyl acetate. The organic phases are washed with brine, dried over sodium sulphate and evaporated. The residue is column chromatographed (silica gel, ethyl acetate followed by ethyl acetate/MeOH 85:15) to yield the desired product as a yellowish oil.

Benzothiazol-2-yl-acetic acid methyl ester
3.2 mL (30 mmol) 2-Amino-thiophenol are dissolved in 100 mL diethylether and treated with 4.17 mL (1 eq.) triethylamine to yield a suspension, to which 3.21 mL (1 eq.) chlorocarbonyl-acetic methyl ester in 10 mL diethylether is added dropwise within 20 min. The resulting suspension is stirred and additional 2 h at room temperature, and the precipitate removed by

filtration. The filtrate is evaporated and column chromatographed (silica gel, ethyl acetate/petroleum ether 1:2) to yield the desired product as a yellowish liquid (5.1 g, 82%).

Alternatively, 3-Benzothiazol-2-yl-7-[4-(2-fluoro-ethyl)-piperazin-1-yl]-chromen-2-one can be prepared as described hereafter:

10 mg (0.025 mmol) 3-benzothiazol-2-yl-7-[4-(2-hydroxy-ethyl)-piperazin-1-yl]-chromen-2-one are dissolved in 3 mL dichloromethane with 2.5 mg (0.8 eq.) 4-dimethylaminopyridine and 0.013 mL (3 eq.) diisopropylethylamine, and cooled to 0°C. 5.7 mg (1.2 eq.) tosyl chloride are added and the reaction mixture stirred for one hour before being allowed to reach room temperature. After an additional two hours stirring, the reaction mixture is evaporated, the residue taken up in tetrahydrofurane and treated with 0.2 mL tetrabutylammonium fluoride (1M in THF). After stirring for 30 minutes, the solution is evaporated and the desired product obtained as a yellow powder.
MS(EI+): 410 (M+1).

The preparation of the starting material is described in Example 4.

Example 2: 7-Benzothiazol-2-yl-1-(2-fluoro-ethyl)-1,2,3,4-tetrahydro-5-oxa-1-aza-phenanthren-6-one

110 mg (9.493 mmol) 1-(2-Fluoro-ethyl)-5-hydroxy-1,2,3,4-tetrahydro-quinoline-6-carbaldehyde and 102 mg (1 eq.) benzothiazol-2-yl-acetic methyl ester are heated to reflux in 5 mL benzene and 2 mL acetonitrile for 5 h, in the presence of 0.097 mL (2 eq.) piperidine. The reaction mixture is allowed to reach RT. The product is obtained after filtration and washing with acetonitrile as an orange solid (mp=285°C).
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.85 (s, 1H); 7.98, 7.90 (2d, 2H); 7.44, 7.32 (2t, 2H); 7.35, 6.60 (2d, 2H); 4.61, 3.72 (2dt, 2 CH_2); 3.44, 2.95 (2dt, 2CH_2); 1.99 (m, CH_2);
MS(EI+): 381 (M+1).

The starting materials are prepared as described hereafter:

1-(2-Fluoro-ethyl)-5-hydroxy-1,2,3,4-tetrahydro-quinoline-6-carbaldehyde

380 mg (1.95 mmol) 1-(2-Fluoro-ethyl)-1,2,3,4-tetrahydro-quinolin-5-ol were dissolved in 6 mL DMF. 0.213 mL (1.2 eq.) $POCl_3$ is added within 15 minutes and the reaction mixture stirred for 18h at RT before evaporation. The residue is column chromatographed (silica gel, ethyl acetate/petroleum ether 1:2) to yield 110 mg (25%) of desired product as a red oil.

1-(2-Fluoro-ethyl)-1,2,3,4-tetrahydro-quinolin-5-ol

500 mg (3.35 mmol) 1,2,3,4-Tetrahydro-quinolin-5-ol and 0.325 mL (1.3 eq.) 1-bromo-2-fluoroethane are heated in 10 mL DMF to 60°C for 96 h, in the presence of 0.63 mL (1.1 eq) diisopropylethylamine. The reaction mixture is allowed to reach RT and extracted with water and a mixture of ethyl acetate and petroleum ether. The organic phases are washed with brine, combined, dried over sodium sulphate and evaporated. The residue is column chromatographed (silica gel, ethyl acetate/petroleum ether 1:2) to yield 380 mg of desired product as an oil.

Example 3: 3-(6-Chloro-imidazo[1,2-a]pyridin-2-yl)-8-(2-fluoro-ethyl)-5,6,7,8-tetrahydro-1-oxa-8-aza-anthracen-2-one

200 mg (0.896 mmol) 1-(2-Fluoro-ethyl)-7-hydroxy-1,2,3,4-tetrahydro-quinoline-6-carbaldehyde and 201 mg (1 eq.) (6-chloro-imidazo[1,2-a]pyridin-2-yl)-acetic acid methyl ester in 6 mL benzene and 3 mL acetonitrile are treated with 0.177 mL (2 eq.) piperidine and refluxed for 18 h. The reaction mixture is allowed to reach room temperature and the precipitate filtered, washed with acetonitrile and dried under high vacuum to yield 240 mg (67%) of desired product as a yellow powder.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.59, 8.44,8.16 (3s, 3H); 7.51, 7.15 (2d, 2H); 7.13 (s, 1H); 4.68, 3.68 (2dt, $2CH_2$); 3.50, 2.82 (2dt, $2CH_2$); 1.99 (m, CH_2).

MS(EI+): 398 (M+1).

M.p. = 290°C (decomposition).

The starting materials are prepared as described hereafter.

1-(2-Fluoro-ethyl)-7-hydroxy-1,2,3,4-tetrahydro-quinoline-6-carbaldehyde

290 mg (1.48 mmol) 1-(2-Fluoro-ethyl)-7-hydroxy-1,2,3,4-tetrahydro-quinolin-7-ol are dissolved in 5 mL DMF. 0.15 mL $POCl_3$ are added dropwise at 0°C. The reaction mixture is slowly heated to 50°C, stirred an additional 3h at this temperature, then cooled to RT and

extracted with ethyl acetate and an aqueous saturated solution of sodium bicarbonate. The combined organic phases are washed with brine, dried over sodium sulphate and evaporated. The residue is column chromatographed (silica gel, ethyl acetate/petroleum ether 1:2) to yield 200 mg (60%) desired product as a brown solid.

1-(2-Fluoro-ethyl)-1,2,3,4-tetrahydro-quinolin-7-ol

500 mg (3.35 mmol) 1,2,3,4-Tetrahydro-quinolin-7-ol are dissolved in 8 mL DMF and stirred at 60°C overnight with 0.325 mL (1.3 eq) 1-bromo-2-fluoroethane, in the presence of 0.63 mL (1.1 eq) diisopropylethylamine. The reaction mixture is extracted with 0.1 N aqu. HCl and ethyl acetate. The combined organic extracts are washed with brine, dried over sodium acetate and evaporated. The residue is column chromatographed (silica gel, ethyl acetate/petroleum ether 1:2 to 1:1) to yield 290 mg (44%) desired product as a brown oil.

(6-Chloro-imidazo[1,2-a]pyridin-2-yl)-acetic acid methyl ester

1.28 g (10 mmol) 2-Amino-5-chloropyridine and 1.18 mL (1 eq) 4-chloro-3-oxo-butyric acid methyl ester are heated to 115 °C in 15 mL toluene for 18 h. The resulting brown suspension is evaporated and heated 1 h to 90°C under high vacuum, then cooled to room temperature and stirred in 100 mL dichloromethane, 10 mL saturated aqueous sodium bicarbonate solution and 40 mL water at 0°C for one hour. The organic phase is then separated and evaporated to yield a brownish solid that is column chromatographed (silica gel, ethyl acetate/petroleum ether 4:1) to yield 700 mg (31%) desired product as a beige powder.

Example 4: 3-Benzothiazol-2-yl-7-[4-(2-hydroxy-ethyl)-piperazin-1-yl]-chromen-2-one

240 mg crude 3-benzothiazol-2-yl-7-piperazin-1-yl-chromen-2-one in 10 mL DMF are stirred for 72h at room temperature in the presence of 858 mg (at least 4 eq.) cesium carbonate and 0.103 mL (at least 2 eq.) 2-iodoethanol. The reaction mixture is extracted with ethyl acetate and a saturated solution of sodium carbonate and washed with brine. The combined organic phases are dried with sodium sulphate and evaporated. The residue is column chromatographed (silica gel, dichloromethane/methanol 95:5 + 1% conc. NH_4OH) to yield 70 mg (26%) desired product as an orange solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.97 (s, 1H); 8.03, 7.97 (2d, 2H); 7.56 (1d, 1H); 7.50, 7.39 (2t, 2H); 6.89 (d, 1H); 8.78 (s, 1H); 3.70 (m, CH_2); 3.47, 2.72 (2m, 2x2CH_2); 2.64 (m, CH_2).
MS(EI+): 408 (M+1).

The starting material is prepared as described hereafter:

3-Benzothiazol-2-yl-7-piperazin-1-yl-chromen-2-one

890 mg (5 mmol) 3-Piperazin-1-yl-phenol are suspended in 100 mL EtOH and treated with 0.42 mL (1 eq.) 12N HCl at a temperateure below 10°C. The suspension ist stirred an hour and slowly evaporated. The remaining beige powder is dried under high vacuum, then taken up in 60 mL DMF. The solution is cooled to 5°C under argon, 0.503 mL (1.1 eq.) $POCl_3$ added dropwise and the reaction mixture stirred for an hour at room temperature, then 30 minutes at 80°C, and finally allowed to cool to room temperature, upon which 2g (3 eq.) solid potassium carbonate is added. The suspension is stirred 30 minutes at room temperature then evaporated, and the residue resuspended and stirred in a 1:1 mixture of dichloromethane and isopropanol. The suspension is filtered, the filtrate evaporated and dried to yield 600 mg crude 2-hydroxy-4-piperazin-1-yl-benzaldehyde. This material is taken up in 40 mL of a 1:1 mixture of benzene and acetonitrile, 2.5 mL (5 eq.) piperidine and 683 mg (0.66 eq.) benzothiazol-2-yl-acetic acid methyl ester are added under argon and the reaction mixture refluxed for an hour. The product is extracted with dichloromethane and brine at 0°C, the combined organic phases dried with sodium sulphate, filtered and evaporated, then triturated with diisopropylether to yield 800 mg desired product (crude).

Example 5:

Staining of APP23 mouse and human alzheimer disease (AD) brain sections using an agent of the invention or Thioflavine S.

Four-micrometer thick paraffin sections from an APP23 mouse at 26 months of age are deparaffinized in xylene and rehydrated. 10 mg of the compound are dissolved in 1 ml DMSO and diluted with deionized water 1:10. This staining solution is applied on sections for about 20 min. Section background is cleared by washing with 95% ethanol. Finally sections are dehydrated in 99% ethanol, cleared in xylene and mounted with Vectashield [tm]. Sections are investigated using fluorescence microscopy with the following filter combination: Excitation 450-490 nm, emission 510 nm. Twenty micrometer thick cryotom sections from a AD brain cortex are air dried and fixated in 4% PFA for 5 min. After washing in tap water

sections are stained either with Thioflavine S or with the compound for 5 min and further processed as described above. The compound is dissolved in DMSO and diluted to a final concentration of 0.01 % with 50% Ethanol, Thioflavine S is dissolved in 50% Ethanol, final concentration is 0.01 %.

In vivo labeling of Aβ in APP23 mice with the agent of the invention

Injection solution is prepared fresh by dissolving 10 mg of the compound in 0.2 mL DMSO diluted with 9.8 ml sterile water. Lower concentrations are prepared by further dilution with water. Four APP23 female mice at 21 month of age receive one single injection of the compound (Injection volume: 1 ml/100gr body weight). The treated animals are killed by decapitation after one hour. The brains are removed and frozen on dry ice. 14 µm thick sections are cut in a cryotome, thawmounted and air-dried. Staining is performed as described above. Sections are analyzed using conventional fluorescence microscopy and confocal microscopy.

Results:

1) Staining of APP23 mice brain sections (which contain amyloid deposits but no neurofibrillary tangles):

The agents of the invention strongly stain amyloid plaques and vascular amyloid deposits in brain sections of APP23 mice.

2) Staining of human AD brain sections (which contain both amyloid deposits and neurofibrillary tangles):

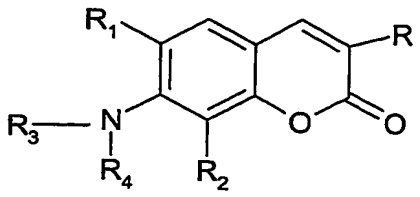
Brain sections taken from frontal cortex of AD patients are stained with the agents of the invention, and the results compared with a Thioflavine S stain. The agents of the invention intensely and selectively stain amyloid deposits and neurofibrillary tangles.

3) Ex vivo staining in APP23 mice:

Intravenous administration of the agents of the invention in APP23 mice leads to a selective and intense staining of amyloid deposits, analyzed ex vivo.

CLAIMS

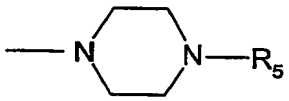
1. A compound of formula I



I

wherein

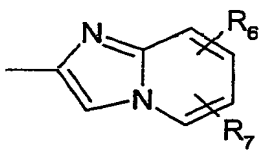
either R_1 and R_2 are both hydrogen and either R_3 and R_4, independently, are H, CH_3, $^{11}CH_3$, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, n being 2, 3 or 4, or R_3 and R_4, together with the nitrogen atom to which they are attached, form a group of formula



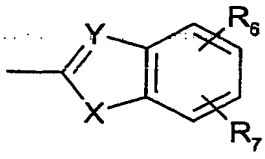
wherein R_5 is H, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, CH_3, $^{11}CH_3$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, n being as defined above,

or one of R_1 and R_2 is hydrogen and the other, together with R_3, forms a $-(CH_2)_m-$ bridge, m being 2 or 3, and R_4 is H, CH_3, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $^{11}CH_3$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, and

R is a group of formula



or

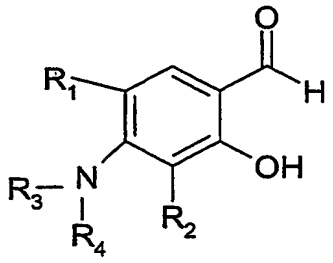


wherein X is O, S or NR_8, R_8 being H, CH_3, $^{11}CH_3$, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$ (n being as defined above), Y is CH or N and R_6 and R_7, independently, are H, NO_2, F, ^{18}F, $O(CH_2)_nF$, $O(CH_2)_n{}^{18}F$, Cl, CN, ^{11}CN, OCH_3, $O^{11}CH_3$, I, ^{123}I, $O(CH_2)_nI$ or $O(CH_2)_n{}^{123}I$ (n being as defined above),

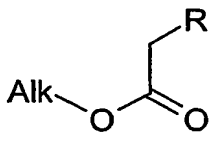
in free base or acid addition salt form, for use as a marker.

2. A process for the production of a compound of formula I as defined in claim 1, or a salt thereof, comprising the step of

a) for the production of a compound of formula I wherein R_3, R_4, R_5, R_6, R_7 and R_8 are different from $^{11}CH_3$, $(CH_2)_n{}^{18}F$, $(CH_2)_n{}^{123}I$, ^{18}F, $O(CH_2)_n{}^{18}F$, ^{11}CN, $O^{11}CH_3$, ^{123}I and $O(CH_2)_n{}^{123}I$, reacting a compound of formula II with a compound of formula III



II



III

wherein R_3 and R_4 as well as R_5 in R_3 and R_4; R_6 and R_7 in R; and R_8 in X are different from $^{11}CH_3$, $(CH_2)_n{}^{18}F$, $(CH_2)_n{}^{123}I$, ^{18}F, $O(CH_2)_n{}^{18}F$, ^{11}CN, $O^{11}CH_3$, ^{123}I and $O(CH_2)_n{}^{123}I$, and Alk is (C_{1-4})alkyl, or

b) for the production of a compound of formula I wherein at least one of R_6 and R_7 is $O^{11}CH_3$, reacting a compound of formula I wherein at least one of R_6 and R_7 is OH with $I^{11}CH_3$ and a base, or

c) for the production of a compound of formula I wherein at least one of R_6 and R_7 is $O(CH_2)_n{}^{18}F$, respectively $O(CH_2)_n{}^{123}I$, reacting a compound of formula I wherein at least one of R_6 and R_7 is $O(CH_2)_nOTs$ or $O(CH_2)_nOMs$ with $^{18}F^{\ominus}$, respectively $^{123}I^{\ominus}$, or

d) for the production of a compound of formula I wherein at least one of R_6 and R_7 is ^{18}F, reacting a compound of formula I wherein at least one of R_6 and R_7 is NO_2 or halogen, with $^{18}F^{\ominus}$, or

e) for the production of a compound of formula I wherein at least one of R_6 and R_7 is ^{123}I, reacting a compound of formula I wherein at least one of R_6 and R_7 is Bu_3Sn, with ^{123}I and hydrogen peroxide, or

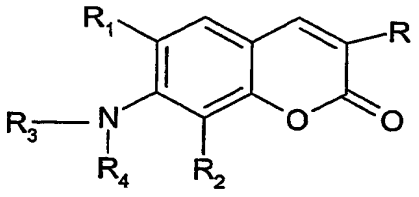
f) for the production of a compound of formula I wherein at least one of R_6 and R_7 is ^{11}CN, reacting a compound of formula I wherein at least one of R_6 and R_7 is OSO_2CF_3 with [^{11}C]cyanide, or

g) for the production of a compound of formula I wherein at least one of R_3, R_4, R_5 and R_8 is $^{11}CH_3$, reacting a compound of formula I wherein at least one of R_3, R_4, R_5 and R_8 is hydrogen, with $^{11}CH_3I$, or

h) for the production of a compound of formula I wherein at least one of R_3, R_4, R_5 and R_8 is $(CH_2)_n{}^{18}F$, respectively $(CH_2)_n{}^{123}I$, reacting a compound of formula I wherein at least one of R_3, R_4, R_5 and R_8 is $(CH_2)_nOTs$ or $(CH_2)_nOMs$ with $^{18}F^{\ominus}$, respectively $I^{\ominus}$,

and recovering the resulting compound of formula I in free base form or in form of an acid addition salt.

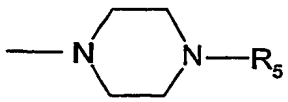
3. A compound of formula I



I

wherein

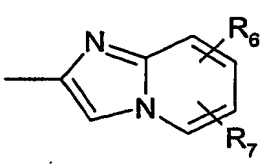
either R_1 and R_2 are both hydrogen and either R_3 and R_4, independently, are H, CH_3, $^{11}CH_3$, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, n being 2, 3 or 4, or R_3 and R_4, together with the nitrogen atom to which they are attached, form a group of formula



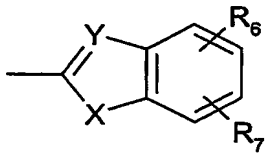
wherein R_5 is H, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, CH_3, $^{11}CH_3$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, n being as defined above,

or one of R_1 and R_2 is hydrogen and the other, together with R_3, forms a $-(CH_2)_m-$ bridge, m being 2 or 3, and R_4 is H, CH_3, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $^{11}CH_3$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, and

R is a group of formula



or



wherein X is O, S or NR_8, R_8 being H, CH_3, $^{11}CH_3$, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$ (n being as defined above), Y is CH or N and R_6 and R_7, independently, are H, NO_2, F, ^{18}F, $O(CH_2)_nF$, $O(CH_2)_n{}^{18}F$, Cl, CN, ^{11}CN, OCH_3, $O^{11}CH_3$, I, ^{123}I, $O(CH_2)_nI$ or $O(CH_2)_n{}^{123}I$ (n being as defined above),

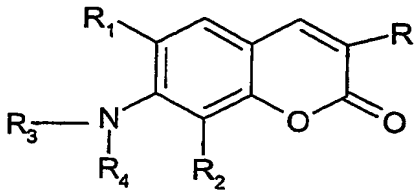
with the exception of

7-Dimethylamino-3-(1-methyl-1H-benzoimidazol-2-yl)-chromen-2-one
3-(1H-Benzoimidazol-2-yl)-7-dimethylamino-chromen-2-one
3-(6-Chloro-benzothiazol-2-yl)-7-dimethylamino-chromen-2-one
3-Benzothiazol-2-yl-7-dimethylamino-chromen-2-one
3-Benzooxazol-2-yl-7-dimethylamino-chromen-2-one
3-Benzooxazol-2-yl-7-methylamino-chromen-2-one
3-(5-Chloro-benzooxazol-2-yl)-7-dimethylamino-chromen-2-one

in free base or acid addition salt form.

4. The compound according to claim 3 which is 3-benzothiazol-2-yl-7-[4-(2-fluoro-ethyl)-piperazin-1-yl]-chromen-2-one, in free base or acid addition salt form.

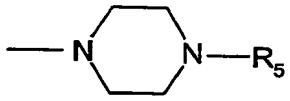
5. A composition for labeling histopathological structures in vitro or in vivo, comprising a compound of formula I



I

wherein

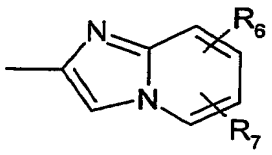
either R_1 and R_2 are both hydrogen and either R_3 and R_4, independently, are H, CH_3, $^{11}CH_3$, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, n being 2, 3 or 4, or R_3 and R_4, together with the nitrogen atom to which they are attached, form a group of formula



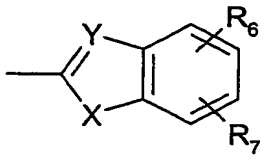
wherein R_5 is H, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, CH_3, $^{11}CH_3$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, n being as defined above,

or one of R_1 and R_2 is hydrogen and the other, together with R_3, forms a $-(CH_2)_m-$ bridge, m being 2 or 3, and R_4 is H, CH_3, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $^{11}CH_3$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, and

R is a group of formula



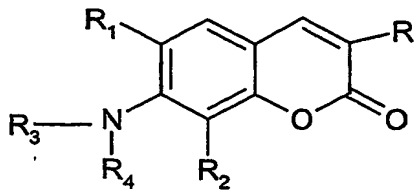
or



wherein X is O, S or NR_8, R_8 being H, CH_3, $^{11}CH_3$, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$ (n being as defined above), Y is CH or N and R_6 and R_7, independently, are H, NO_2, F, ^{18}F, $O(CH_2)_nF$, $O(CH_2)_n{}^{18}F$, Cl, CN, ^{11}CN, OCH_3, $O^{11}CH_3$, I, ^{123}I, $O(CH_2)_nI$ or $O(CH_2)_n{}^{123}I$ (n being as defined above),

in free base or acid addition salt form.

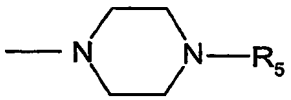
6. A method for labeling histopathological structures in vitro or in vivo, comprising contacting brain tissue with a compound of formula I



I

wherein

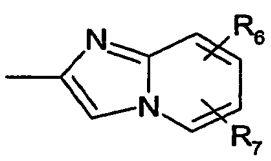
either R_1 and R_2 are both hydrogen and either R_3 and R_4, independently, are H, CH_3, $^{11}CH_3$, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, n being 2, 3 or 4, or R_3 and R_4, together with the nitrogen atom to which they are attached, form a group of formula



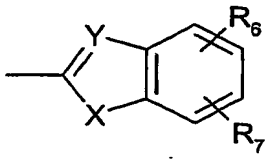
wherein R_5 is H, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, CH_3, $^{11}CH_3$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, n being as defined above,

or one of R_1 and R_2 is hydrogen and the other, together with R_3, forms a $-(CH_2)_m-$ bridge, m being 2 or 3, and R_4 is H, CH_3, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $^{11}CH_3$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$, and

R is a group of formula



or



wherein X is O, S or NR_8, R_8 being H, CH_3, $^{11}CH_3$, $(CH_2)_nI$, $(CH_2)_n{}^{123}I$, $(CH_2)_nOH$, $(CH_2)_nF$ or $(CH_2)_n{}^{18}F$ (n being as defined above), Y is CH or N and R_6 and R_7, independently, are H, NO_2, F, ^{18}F, $O(CH_2)_nF$, $O(CH_2)_n{}^{18}F$, Cl, CN, ^{11}CN, OCH_3, $O^{11}CH_3$, I, ^{123}I, $O(CH_2)_nI$ or $O(CH_2)_n{}^{123}I$ (n being as defined above),

in free base or acid addition salt form.

7. A method according to claim 6, for labeling ß-amyloid plaques and neurofibrillary tangles.

8. A method according to claim 6 or 7, comprising administering the compound of formula I to a patient.

9. A method according to any of claims 6 to 8, comprising the further step of determining whether the compound of formula I labeled the target structure.

10. A method according to claim 9, comprising observing the target structure labeled with a non-radioactive compound of formula I, using fluorescence microscopy.

11. A method according to claim 9, comprising observing the target structure labeled with a radioactive compound of formula I, using positron emission tomography (PET).

12. A method according to claim 9, comprising observing the target structure labeled with a radioactive compound of formula I, using single photon emission computed tomography (SPECT).

13. A method according to any one of claims 6 to 9, 11 and 12, for diagnosing Alzheimer's disease.

14. A method according to claim 13, for monitoring the effectiveness of a therapeutic treatment of Alzheimer's disease.

15. A method according to any of claims 6, 7, 9 and 10, for detecting histopathological hallmarks of Alzheimer's disease.

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☑ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☑ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☑ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.